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(54) A process for steeping cereals with a new enzyme preparation.

(57) A process for steeping corn or sorghum kernels in warm water containing sulfur dioxide in the presence of an enzyme preparation comprising one or more phytin degrading enzymes, preferably in such an amount that the phytin present in the corn or sorghum is substantially degraded. The enzyme preparation may comprise phytase and/or acid phosphatase and optionally other plant material degrading enzymes. The steeping time may be 12 to 18 hours. The steeping may be interrupted by an intermediate milling step, reducing the steeping time.

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A process for steeping cereals with a new enzyme preparation

The invention relates to a process for steeping corn or sorghum kernels in warm water containing sulfur dioxide. Hereinafter for convenience we will only mention corn. Steeping of corn kernels is the first step in the processing of corn to obtain different product fractions like germs, proteins and starch. In this first step the hard corn kernels are steeped to soften them. The kernels absorb water and they swell. At the same time water-soluble substances are leached out of the corn and pass into the steepwater. The temperature of the steepwater is mostly 40-55 °C. The sulfur dioxide which in general is present for about 0.2%, breaks the cell wall structure and prevents the growth of microorganisms during steeping. The steeping process lasts about 48 hours. All subsequent steps, in which the different product fractions are obtained, are much shorter. The corn steep liquor (CSL) obtained is concentrated by evaporation. The product obtained will mainly be used as animal feed but also as a nutrient in microbial fermentations. The swollen kernels are further separated in germ, fiber, starch and protein fractions in different steps.

Just like in many other plant seeds phytic acid, the hexaphosphate ester of myoinositol, is present in the corn kernels. Phytic acid mostly appears in the form of calcium and magnesium salts, which in general are called phytin. A large part of the phosphorus present in plants is stored in these compounds. In the steeping process the largest part of the phytic acid comes in the CSL. It forms an undesirable component therein for the following reasons.

Phytic acid in CSL deposits a sludge with proteins and metal ions. This has caused problems in concentrating by evaporation, transporting and storing the CSL.

When used as a nutrient in microbial fermentations, CSL is diluted and the pH is raised to 4-5. When this medium is sterilized the phytic acid forms a precipitate coating on the inner surface of the fermentor. This precipitate is hard to scrub off afterwards and it also interferes with the purification of the fermentation end products.

When CSL is used as animal feed the phytic acid present gives the following problems. Phytic acid, because it interacts with multivalent metal ions, interferes with the assimilation of various metals in the body of animals (and humans). This may lead to deficiency disorders. Phytic acid would also inhibit various enzymes in the body such as pepsin. Besides, the phosphate present in the phytic acid is not available for monogastric animals, because they only can digest phytic acid to a restricted extent.

US patent specification 2,515,157 describes a process for the treatment of CSL to obtain an improved nutrient for antibiotic fermentations. In this process the phytic acid is removed by adding an aluminium ions furnishing compound to the CSL at low pH, heating and separating the aluminium phytate formed.

US patent specification 2,712,516 describes a similar process wherein phytate is precipitated as its calcium salt. The processes described in these US patent specifications are performed after the steeping process. Therefore, an additional step is required for removing phytic acid.

Now it has been found that this additional step can be avoided by performing the steeping in the presence of an enzyme preparation comprising one or more phytin degrading enzymes.

This invention provides in particular a process for processing corn or sorghum, which comprises the consecutive steps of

a) steeping corn or sorghum kernels in warm water containing sulfur dioxide in the presence of an enzyme preparation comprising one or more phytin degrading enzymes,

b) separating the steepwater from the kernels and concentrating it,

c) milling the kernels coarsely and separating and dewatering germs,

d) fine-milling the kernels, separating fibers from starch and protein, and dewatering the fiber fraction, and

e) separating starch and protein from each other, concentrating the protein fraction and drying and/or converting the starch fraction.

Preferably the enzyme preparation comprises such an amount of one or more phytin degrading enzymes that the phytin present in the kernels is substantially degraded. With the term "phytin" used herein the salts of phytic acid and also phytic acid itself are meant.

Phytin degrading enzymes dephosphorylate inositol phosphates to yield inositol and orthophosphate. Phytin degrading enzymes include phytase and acid phosphatases. Phytase and acid phosphatases are produced by various microorganisms like Aspergillus spp., Rhizopus spp. and yeasts (Appl. Microbiol 16 (1968) 1348-1357; Enzyme Microb. Technol. 5 (1983), 377-382) while phytase is also produced by various plant seeds, as for example wheat, during germination. Phytin degrading enzymes are very active at the low

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pH of the steepwater. According to methods known in the art enzyme preparations can be obtained from the above mentioned organisms. It is found that phytin in corn is degraded most efficiently with enzymes from Aspergillus spp. Thus,at the same enzyme dosage an Aspergillus niger enzyme preparation is more efficient than wheat phytase.

Microbially produced enzyme preparations may comprise additional plant material degrading enzymes such as enzymes having cellulase, hemicellulase and/or pectinase activity. These other activities contribute to the advantages which are obtained by the process of the invention. Suitable enzyme preparations are for example enzymes of the Econase EP 43 series manufactured by Alko Ltd.

The temperature during the steeping process according to the invention is 20-60°C, and generally about 50°C. The applied amount of enzyme preparation depends on the preparation used, the phytin contents of the corn kernels and the reaction conditions. The right dosage can easily be estimated by a person skilled in the art.

The process according to the invention offers, besides avoiding an additional step, various important advantages. First, by adding the enzyme preparation the steeping process is accelerated to such an extent that the steeping time may be reduced considerably. Since the steeping process is the longest step in total corn processing, a reduction thereof is of great economical importance. Thus the steeping process may be reduced to only 12 hours without any losses in the main product fraction yields. Preferably steeping time will be 12-18 hours, however, longer steeping times up to 48 hours are possible.

Secondly, the separation processes after the steeping process according to the invention are improved and give higher yields. When steeping is performed for e.g. 16 hours in the presence of the enzyme preparation, the yield of starch is higher than in the case of the conventional steeping process.

Thirdly, steeping corn in the presence of phytin degrading enzymes leads to corn steep liquor that does not contain phytin. As a result concentration of CSL is easier and the product obtained is excellently suitable for animal feed and for microbial fermentations.

The steeping time can yet be further reduced by performing the steeping process in two steps, first steeping for 4-10 hours, followed by milling the corn kernels and then further steeping the milled corn kernels for another 3-6 hours. Preferably the second stage of this double stage steeping is carried out in water not containing sulfur dioxide.

In the examples the process of the invention is carried out on laboratory scale by standard Pelshenke and Lindemann determination. As may be expected, the results obtained when carrying out the process industrially will be similar or even better due to improved separating techniques.

Example I

In a number of tests 50 g of corn kernels are steeped in water of 50°C containing 0.2% sulfur dioxide, in the presence or in the absence of an amount of Econase EP 434. This enzyme preparation has as major activities phytin and cellulose degrading activities and as minor activities hemicellulase and pectinase. The steeping times of the tests vary of from 12 to 48 hours, as shown in table A.

The enzyme dosages are presented as Phytin degrading units / g of corn. One phytin degrading unit (1 PU) is the amount of enzyme which liberates 1 nmol of inorganic phosphorus from sodium phytate per minute under standard conditions (40°C, pH 5,5). The kernels after steeping are processed further to obtain the product fractions mentioned in Table B.

TABLE A

Test	1	2	3	4	5	6
steeping time (h)	48	48	24	20	16	12
dosage of Econase EP 434 (PU/g corn)	-	70	135	160	200	270

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TABLE B

Results of single stage steeping						
	Yield in % of dry weight					
Test	1	2	3	4	5	6
dry substance in CSL	5.28	5.61	4.78	4.72	4.23	3.91
germs	7.34	7.12	7.42	7.66	9.00	7.51
fibers (starch content)[a]	9.70 (19.01)	9.21 (17.16)	9.55 (16.91)	9.52 (8.60)	9.41 (16.71)	9.70 (17.46)
starch (protein content)[a]	64.09 (0.37)	65.49 (0.37)	65.29 (0.35)	65.38 (0.43)	66.20 (0.39)	64.00 (0.44)
gluten (protein content)[a]	7.31 (46.57)	6.24 (51.43)	7.57 (47.52)	8.12 (49.21)	6.94 (57.60)	9.42 (42.76)
dry substance in supernatant	2.21	2.25	2.88	2.89	3.00	2.59
starch recovery	94.4	96.5	96.2	96.3	97.5	94.3
total dry substance recovery	95.91	95.92	97.49	98.29	98.78	97.15

a) expressed as % of the fraction

It appears from Table B that the starch yield after 16 or 48 hours of single stage steeping in the presence of the enzyme preparation is higher than in the case of conventional steeping without enzyme preparation, and after 12 hours of steeping in the presence of the enzyme preparation the starch yield is almost as high as in the case of conventional steeping without enzyme preparation.

Example II

50 g of corn kernels are presteeped in water of 50 °C containing 0.2% sulfur dioxide and Econase EP 434 providing 135 PU / g of corn for 6 hours. Following manual degermination the product is milled coarsely. Then the germs are added back to the slurry. Thereafter the second stage of the steeping is carried out in fresh water of 50 °C containing Econase EP 434 providing 135 PU / g of corn for 4 hours. The suspension obtained is processed further to obtain the product fractions mentioned in Table C.

TABLE C

	Yield in % of dry weight
dry substance in CSL	2.19
germs	8.80
fibers (starch content)	9.64 (20.99)
starch (protein content)	65.53 (0.37)
gluten (protein content)	6.8 (56.74)
dry substance in supernatant	5.45
starch recovery	96.5
total dry substance recovery	98.41

Note:

In this test it is necessary to degerminate before milling because the mill used would damage the germ. When the double stage steeping is carried out industrially a mill would be used which will not damage the germ. Degermination is not necessary then.

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Example III

CSL is diluted 1:10 and the pH is adjusted to 5.0. Corn flour is diluted 1:10 with 0.2 M citrate buffer pH 5.0. Sodium azide is added at a concentration of 0.02% to inhibit microbial growth. Aspergillus spp. enzyme preparation containing phytin degrading activity or wheat phytase (Sigma P-1259) is added at a dosage of 7000 PU/ gram of phytin (300 PU per each ml of CSL dilution and 150 PU per each 2 grams of corn flour).

Suspensions are incubated in a shaker (250 rpm) at 50°C. At fixed intervals the reaction is stopped with equal volume of 6% (w/v) H_2SO_4. Phytate is extracted to the acidic liquid for 30 min. at room temperature. Phytic acid is then precipitated from a clear supernatant with ferric chloride. Ferric ions are removed by precipitation with sodium hydroxide. Phytate is determined by HPLC using sodium phytate as a standard.

Table D shows the residual phytin content of CSL and corn flour after incubation with phytin degrading enzymes. In experiment a) incubation is carried out with Aspergillus spp. enzyme preparation, and in experiment b) incubation is carried out with wheat phytase.

TABLE D

Comparing Aspergillus spp. enzyme preparation and wheat phytase.					
Substrate	Incubation time (h)	Phytin (as phytic acid)			
		exp. a)		exp. b)	
		mg/ml	%	mg/ml	%
CSL	0	3.1	100	3.4	100
	2	2.7	87	2.4	71
	4	1.4	45	1.9	56
	10	1.0	32	2.0	59
	24	0	0	1.4	41
corn flour	0	13.6	100	11.4	100
	2	9.1	67	9.1	80
	4	0	0	7.9	69
	10	0	0	6.8	60
	24	0	0	2.3	20

Table D shows that phytic acid content can be reduced considerably with both phytin degrading enzymes. At the same enzyme dosage Aspergillus spp. enzyme preparation is more efficient than wheat phytase.

Example IV

25 g of corn kernels are steeped in 50 ml water of 50°C containing 0.2% sulfur dioxide. In the control no enzyme preparation is added and in the test according to the invention an Aspergillus spp. enzyme preparation is added at a dosage of 135 PU/g corn. Steeping time is 24 hours or 48 hours.

After steeping an amount of CSL is extracted for 30 min. with an equal volume of 6% (w/v) H_2SO_4 at room temperature. Phytic acid is precipitated from a clear supernatant with ferric chloride. Ferric ions are removed by precipitation with sodium hydroxide. Phytate is determined by HPLC using sodium phytate as a standard.

Table E shows the amount of phytic acid in CSL. Experiment a) comprises conventional steeping without phytin degrading enzymes and experiment b) comprises steeping in the presence of the above enzyme preparation.

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TABLE E

Phytin content of CSL		
steepding time (h)	mg phytic acid / ml CSL	
	exp. a)	exp.b)
24	1.6	0
48	3.1	0

Table E shows that when corn kernels are steeped in the presence of phytin degrading enzymes CSL is free from phytin.

Example V

Econase EP 434 and a plant cell wall degrading enzyme preparation with negligible phytin degrading activity are tested in one-step and in two-step steeping.

In one-step steeping 50 g of corn kernels are steeped in water of 50°C containing 0.2% sulfur dioxide. The dosage of Econase EP 434 is 135 PU/g corn. Equal volume of the plant cell wall degrading enzyme preparation with negligible phytin degrading activity is applied. Steeping time is 20 hours. The kernels are processed further according to Pelshenke and Lindemann method.

In two-step steeping 50 g of corn kernels are pre-steeped for 6 hours in water of 50°C containing 0.2% sulfur dioxide and Econase EP 434 providing 135 PU/g corn or an equal volume of plant cell wall degrading enzyme preparation with negligible phytin degrading activity. Following manual degermination the product is milled coarsely. Then the germs are added back to the slurry. Thereafter the second stage of the steeping is carried out for 4 hours in fresh water of 50°C containing Econase EP 434 providing 135 PU/g corn or an equal volume of plant cell wall degrading enzyme preparation with negligible phytin degrading activity. The slurry is further processed according to Pelshenke and Lindemann method.

TABLE F

Starch recoveries with different enzyme preparations.			
1. Econase EP 434 2. plant cell wall degrading enzyme preparation with negligible phytin degrading activity.			
Steeping process	Steeping time h	enzyme	starch recovery %
One-step	20	1	97.0
	20	2	94.4
Two-step	6 + 4	1	96.5
	6 + 4	2	91.4

It appears from Table F that the starch yield is higher when the kernels are treated with an enzyme preparation containing phytin degrading activity.

Claims

1. A process for steeping corn or sorghum kernels in warm water containing sulfur dioxide, characterized in that the steeping is performed in the presence of an enzyme preparation comprising one or more phytin degrading enzymes.

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2. A process according to claim 1, characterized in that the enzyme preparation contains such an amount of one or more phytin degrading enzymes that the phytin present in corn is substantially degraded.

3. A process according to claim 1 or 2, characterized in that the enzyme preparation comprises phytase and/or acid phosphatase.

4. A process according to any one of claims 1-3, characterized in that the enzyme preparation additionally comprises other plant material degrading enzymes.

5. A process according to claim 4, characterized in that the plant material degrading enzymes possess cellulase, hemicellulase and/or pectinase activity.

6. A process according to any one of claims 1-5, characterized in that the phytin degrading enzyme preparation is obtained from wheat or Aspergillus spp. or from other plant or microbial sources.

7. A process according to any one of claims 1-6, characterized in that the temperature of the water is 20-60° C.

8. A process according to any one of claims 1-7, characterized in that the corn or sorghum kernels are steeped for 12-48 hours.

9 A process according to claim 8, characterized in that the kernels are steeped for 12-18 hours.

10. A process for processing corn or sorghum, which comprises the consecutive steps of

a) steeping corn or sorghum kernels in warm water containing sulfur dioxide,

b) separating the steepwater from the kernels and concentrating it,

c) milling the kernels coarsely and separating and dewatering germs,

d) fine-milling the kernels, separating fibers from starch and protein, and dewatering the fiber fraction, and

e) separating starch and protein from each other, concentrating the protein fraction and drying and/or converting the starch fraction, characterized in that the steeping in step a) is performed according to one of claims 1-9.

11. A process according to claims 1-10, characterized in that the steeping is performed in two steps, first steeping for 4-10 hours followed by milling the kernels and then further steeping the milled kernels for another 3-6 hours.

12. A process according to claim 11, characterized in that the second stage of the steeping is carried out in water not containing sulfur dioxide.

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EUROPEAN SEARCH REPORT

Application Number

EP 88 20 2394

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
Y	US-A-2 555 235 (E.G. HUZENLAUB et al.) * Column 1, line 54 - column 2, line 18; column 4, lines 58-64; column 5, lines 35-59 *	1	A 23 L 1/105 A 23 L 1/015
A		2-5	

Y	EP-A-0 156 174 (GALAM LTD et al.) * Claim 1 *	1	
A	* Claims 9,10 *	7-9	

A	US-A-3 966 971 (A.L. MOREHOUSE et al.) * Claim 1; column 1, line 36 - column 2, line 11; column 9, line 35 - column 10, line 11 *	1-3	

P,A	EP-A-0 267 637 (DORR-OLIVER et al.) * Claims 1,2 *	1,4,5	

A	JOURNAL OF INDUSTRIAL MICROBIOLOGY, vol. 2, no. 3, October 1987, pages 195-200, Society for Industrial Microbiology, Amsterdam, NL; Y.W. HAN et al.: "Phytase production by Aspergillus ficuum on semisolid substrate" * Page 195: "Summary"; page 196, right-hand column, last paragraph - page 197, left-hand column, first half; page 199, table 3, left-hand column, last but one paragraph *	1-3,6	TECHNICAL FIELDS SEARCHED (Int. Cl.4) A 23 L C 08 B A 23 J A 23 K C 12 N
	--- -/-		

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	15-03-1989	DEKEIREL M.J.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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D : document cited in the application
L : document cited for other reasons

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A	CHEMICAL ABSTRACTS, vol. 68, no. 11, 11th March 1968, page 4666, abstract no. 48355a, Columbus, Ohio, US; H. MLODECKI et al.: "Influence of culinary processes on content of phytin compounds in some groats and flakes", & ROCZ. PANSTW. ZAKL. HIG. 18(5). 605-610(1967) * Abstract * ---	1	
A	JOURNAL OF FOOD SCIENCE, vol. 48, 1983, pages 953,954,985, Chicago, US; Y. LOPEZ et al.: " Release of phosphorus from phytate by natural lactic acid fermentation" * Page 953, left-hand column, last 2 lines - page 954, left-hand column, table 2; page 985, left-hand column, paragraphs 2,3 * -----	1	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)

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THE HAGUE	15-03-1989	DEKEIREL M.J.

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